JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. 2010, 58, 10109–10117 10109
DOI:10.1021/jf102400t

Stability of Lutein in Wholegrain Bakery Products Naturally High in Lutein or Fortified with Free Lutein

El-Sayed M. Abdel-Aal,* J. Christopher Young, Humayoun Akhtar, and Iwona Rabalski

Guelph Food Research Centre, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, Ontario, Canada N1G 5C9

Lutein is a yellow pigment found in common foods that promotes the health of eyes and skin and is associated with reduced risk of age-related macular degeneration and cataracts. In the present study, selected high-lutein wheat and corn were milled into wholegrain flours by two mills to improve flour uniformity. The high-lutein and lutein-fortified wholegrain flours were processed into breads, cookies, and muffins to study lutein stability during baking and subsequent storage. Lutein and its isomers were separated, identified, and quantified by LC-UV/vis and LC-MS following extraction with water-saturated 1-butanol. Baking resulted in a significant reduction in *all-trans*-lutein and the formation of *cis*-lutein and *cis*-zeaxanthin isomers. Subsequent storage at ambient temperature had a slight impact on the content of *all-trans*-lutein. Effects of processing were more pronounced in lutein-fortified products, and the degradation rate of lutein was influenced by concentration and baking recipe. Fortified cookies and muffins showed greater lutein reduction compared with bread. Despite the significant reduction in lutein, the fortified bakery products still possessed reasonable amounts per serving that would enhance daily intake and consumption of wholegrain foods.

KEYWORDS: Lutein; zeaxanthin; stability of lutein; bread; cookie; muffin

INTRODUCTION

Lutein, a dihydroxylated carotenoid, plays significant roles in promoting the health of eyes and skin and in reducing the risk of age-related macular degeneration (AMD) (*1*), cataracts (*2*), cancer (*3*), and cardiovascular disease (*4*). Lutein and zeaxanthin (another dihydroxylated carotenoid) constitute the pigments found in the yellow spot of the human retina (*5*), and they provide several protective functions; that is, they protect the macula from damage by blue light (*6*), improve visual acuity (*7*), and scavenge harmful reactive oxygen species (*8*). The difference between lutein and zeaxanthin is in the type of ionone ring; lutein contains a β-ionone ring and an ε-ionone ring, whereas zeaxanthin has two β-ionone rings. In general, carotenoids are essential for human health and must be provided in the diet, and thus their abundance in the human body is entirely dependent on dietary intake.

Lutein is found at relatively high content in some commonly consumed foods such as spinach, kale, and eggs. In our previous studies, several wheat species including durum, einkorn, Kamut, and Khorasan and yellow corn were identified as promising ingredients for the development of high-lutein functional foods on the basis of their relatively higher level of lutein compared with spelt and soft and hard wheat species (*9*, *10*). In high-lutein wheat species, lutein ranges from 5.4 to 7.4 μg/g, but corn is exceptionally high in lutein at an average level of 21.9 μg/g. Kean et al. (*11*) also showed that lutein and zeaxanthin are the major carotenoids in corn milled fractions and account for about 70% of the total carotenoids. This makes corn a promising blending flour ingredient in the development of high-lutein functional foods. In addition to lutein, grains contain small concentrations of zeaxanthin and β-cryptoxanthin and trace amounts of their *cis*-isomers (*10*). Lutein content also varied considerably in einkorn accessions and varieties, ranging between 6.4 and 13.4 μg/g with an overall average 8.4 μg/g (*12*). Einkorn was also found to contain about twice as much lutein as durum, which had more than twice the lutein compared with bread wheat (*13*).

Lutein is also present at very high concentration in some plant materials such as marigold flowers, the main commercial source of dietary lutein supplement (*14*, *15*). Several food-grade lutein products are now made from marigold flowers in either free or bound (esterified with long chain fatty acids) form. These products could be used to boost lutein content in foods through food fortification and/or dietary supplements. Both lutein forms are absorbed from foods and dietary supplements, but the ester form requires prior de-esterification by intestinal enzymes (*16*). Recently, a method for the thorough identification and quantification of lutein regioisometric esters has been developed in our laboratory (*17*). We identified 17 lutein regioisomeric mono- and diester compounds on the basis of their LC-UV/vis, LC-MS, and NMR properties compared with those of synthetic lutein esters. This method would be critical to study the stability and efficacy of lutein in fortified foods and dietary supplements. The present study is a continuation of our effort to develop high-lutein wholegrain functional foods based on using naturally high-lutein grain materials and lutein-fortified grain flours. During the course of this study, naturally high-lutein and lutein-fortified whole wheat breads, cookies, and muffins were formulated and used to investigate lutein stability in these products.

*Corresponding author [phone (519) 780-8031; fax (519) 829-2600; e-mail elsayed.abdelaal@agr.gc.ca].

Published 2010 by the American Chemical Society

Published on Web 08/24/2010

An authentic free lutein product was utilized in the fortification process. The stability of lutein and the formation of *cis*-isomers during baking and subsequent storage at ambient temperature were investigated using LC-UV/vis and LC-MS techniques. The quality of the high-lutein bakery products was also evaluated on the basis of objective and subjective measurements. The development of high-lutein staple foods would be of interest to the food industry to enhance lutein intake.

MATERIALS AND METHODS

Wheat and Corn Materials. The materials used in this study include an einkorn wheat (*Triticum monococcum* L.) cultivar known to have high content of lutein, AC Knowles, Khorasan (*Triticum turgidum* spp. *turanicum*) accession PI211691, durum (*Triticum turgidum* spp. *durum*) cultivar Kyle, bread wheat cultivar Katepwa, commercially prepared whole wheat bread flour, and commercially prepared pastry flour. The commercial flours were purchased from a local market in Guelph, ON, Canada. The wheat grains were obtained from plots grown at the experimental farm of the University of Saskatchewan, Saskatoon, SK, Canada, except for einkorn AC Knowles, which was obtained from the Eastern Cereals and Oilseeds Research Centre, Ottawa, ON, Canada. A commercial normal yellow corn (*Zea mays*) meal sample was included in the study as the partial replacement of wheat due to the higher content of lutein compared with einkorn flour and was purchased along with the whole wheat bread flour. Becel margarine, commercial dry yeast, baking powder, and sugar were purchased from the retail market in Guelph, ON, Canada.

The wheat flours were prepared as described in our earlier study (*10*). Immediately after harvest, the grains were dried to approximately 10% moisture content. The hulled einkorn grains were dehulled by passing the grains between a pair of rubber-coated rollers followed by air aspiration. The dehulled (hulls removed by rollers) and hulless (free-threshing) grains were ground on a Cyclone sample mill (Udy Co., Fort Collins, CO) equipped with a 500 μm screen to obtain whole wheat flour for compositional analyses. For baking purposes the wheat grains were milled into wholegrain flours using a combination of two milling systems, a roller mill (Brabender Co., South Hackensack, NJ) and a laboratory mill (IKA Works Inc., Wilmington, NC) to reduce the size of coarse bran fraction, followed by an additional sifting through a 0.355 mm sieve on an RX-29 RO-Tap shaker (W. S. Tylor, Mentor, OH). The wholegrain flours were thoroughly mixed to ensure uniformity and kept at 4 °C until processing and analysis. The commercial corn meal was passed through a Udy mill equipped with a 0.5 mm sieve and then through a 0.335 mm sieve prior to processing. The wholegrain flours were fortified with free lutein powder (85% pure) at a level of 4.7 mg/100 g of flour and thoroughly mixed.

Preparation of Bakery Products. Four bakery products including pan bread, flat bread, cookies, and muffins were prepared with high-lutein and lutein-fortified whole wheat flours. A series of baking trials was initially conducted to optimize baking formulas and conditions. Lutein fortification was performed to achieve a level of about 1.0 mg of free lutein/serving of baked product (30 g). Due to the sensitivity of lutein to oxidation and isomerization during baking process, flours were fortified at higher levels than the targeted level (1.0 mg/serving) by about 40% more to compensate for the anticipated loss of lutein during thermal processing. Pan bread was prepared according to the optimized straight dough breadmaking of the approved method of the AACC, method 10-10B (*18*), with some modifications. The baking formula of fortified and unfortified bread includes 100 g of flour, 60 ± 3 g of water, 2 g of quick yeast, 1.5 g of salt, 0.1 g of sugar, and 5 g of gluten. Of 28 pan bread formulas being baked, 3 formulas were chosen for further investigation on the basis of loaf volume, texture uniformity, and taste. These formulas are control (100% whole wheat flour), a blend of whole wheat and einkorn flours (1:1), and a blend of whole wheat, einkorn, and corn flours (5:4:1). Baking time was 25 min at 175 °C.

One-layer flat bread was prepared using the bulk dough method as described by Abdel-Aal et al. (*19*) but without fermentation. A basic baking formula (fortified and unfortified) was used in the preparation of flat bread containing only flour, salt, and water. The amount of water was variable, approximately 50.5 ± 5 g, depending on type of flour. It was necessary to adjust the amount of water for each of the flours to obtain proper dough elasticity during rolling and handling. Four baking formulas including 100% einkorn, 100% Khorasan, 100% durum, and durum/corn (1:1) were prepared as unfortified and fortified forms and baked into flat bread. No fermentation was conducted to obtain one-layer crispy flat bread. Baking was performed at 250 °C for 5 ± 1 min.

The unfortified and fortified cookies were prepared on the basis of the modified AACC method 10-50D (*18*) to obtain desirable color, crispiness, and taste. The sugar content was reduced from 57.8 to 40% in accordance with flour base (100 g of flour, 40 g of sugar, 1 g of salt, 1 g of sodium bicarbonate, 10 g of water). Three cookie formulas based on 100% commercial pastry flour, 100% einkorn, and 1:1 einkorn/corn were prepared as unfortified and fortified with lutein. Baking was performed at 175 °C for 13 min.

Because there is no official AACC procedure for the preparation of muffins, an in-house baking method was used in muffin-making. Several baking formulas were initially prepared and evaluated with regard to baking performance and sensory properties (texture, color, and taste). The base recipe was 100 g of flour, 62.5 g of sugar, 1.25 g of salt, 2.5 g of baking powder, 22.9 g of margarine, and 83.3 g of water. A mixture of einkorn and corn flour at a ratio of 1:1 was used as the base flour (fortified and unfortified) in making muffins. Baking was done at 175 °C for 30 min.

All bakes were performed in at least five trials and left for about 1 h at room temperature before being evaluated or stored to study stability of lutein over storage at room temperature. High-moisture muffin and pan bread products were stored for 3 and 5 days, respectively, whereas flat breads and cookies were stored for up to 8 weeks. The products were packed into plastic bags and stored at room temperature. The quality of bakery products was based on the measurement of physical and sensory properties. Physical properties include loaf volume and weight for pan bread, symmetry and thickness for flat bread, and spread factor for cookies. Sensory properties and acceptability were assessed by three expert panels. For carotenoid analysis all baked products were prepared according to AACC method 62-05 (*18*) and kept frozen at −20 °C until extraction and analysis.

Analysis of Lutein and Other Carotenoids. Grain flours and bakery products were extracted with water-saturated 1-butanol for the determination of lutein and other carotenoids. This solvent was more effective in extracting carotenoids from wheat compared with 80% aqueous ethanol, 80% aqueous methanol, methyl *tert*-butyl ether, and tetrahydrofuran (*10*). Approximately 0.5−1.0 g of sample was homogenized in 10 mL of solvent for 30 s at 5000 rpm in a PT 10-35 Polytron homogenizer (Kinematica AG, Switzerland), kept for 30 min at room temperature, and homogenized again for 30 s. The mixture was centrifuged at 10000*g* for 5 min, and an aliquot of the supernatant (0.5 mL) was filtered through a 0.45 μm nylon Acrodisc syringe filter (Pall Gelman Laboratory, Ann Arbor, MI). A few drops (2−3) of the filtrate were first discarded, and the remainder was collected for HPLC and LC-MS analyses. All extraction experiments were performed under dim light, and the extraction tubes were wrapped with black paper to avoid sample degradation by photooxidation.

The carotenoid extracts were separated and quantified by high-performance liquid chromatography (HPLC) using an 1100 series chromatograph (Agilent, Mississauga, ON, Canada) as outlined in our previous study (*10*). The HPLC system was equipped with a model G1311A quaternary pump, a G1329A temperature-controlled injector, a G1316A temperature-controlled column thermostat, a G1322A degasser, a G1315B photodiode array detector (PDA), and a ChemStation v.8.04 data acquisition system with the capability of conducting isoabsorbance plotting and three-dimensional graphic analyses. The separation was performed on a 100 mm × 4.6 mm, i.d., 3 μm short C30 YMC Carotenoid column (Waters, Mississauga, ON, Canada). The column was operated at 35 °C and eluted with a gradient mobile system consisting of (A) methanol/methyl *tert*-butyl ether/nano pure water (81:15:4, v/v/v) and (B) methyl *tert*-butyl ether/methanol (90:10, v/v) at 1 mL/min. The gradient was programmed as follows: 0−9 min, 100−75% A; 9−10 min, 75−0% A; 10−12 min, hold at 0% A; 12−13 min, 0−100% A; and 13−15 min, hold at 100% A for the short column. The separated carotenoids were detected and measured at 450 nm, and the identity of carotenoids was based on the congruence of retention times and UV/vis spectra with those of pure authentic standards.

Four pure authentic carotenoids that are common in grains, *all-trans*-lutein (90% purity) and *all-trans*-β-carotene (95% purity), were purchased from Sigma (Sigma-Aldrich Canada Ltd., Oakville, ON, Canada), and *all-trans*-zeaxanthin (95% purity) and *all-trans*-β-cryptoxanthin (95% purity)

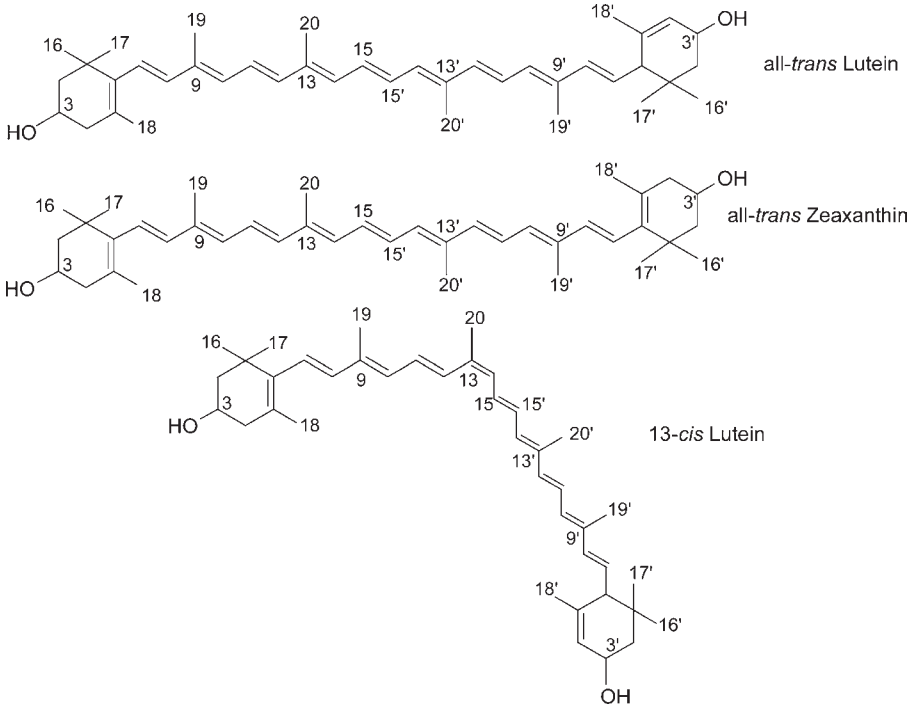

Figure 1. Chemical structures of *all-trans*-lutein, *all-trans*-zeaxanthin, and 13-*cis*-lutein.

were from ChromaDex (Santa Ana, CA). These carotenoid standards were used for identification and quantification. Five concentrations in the range of 4−85 ng per injection (20 μL) were prepared for each carotenoid in butanol and used to check linearity, to optimize the analytical method, and to generate regression equations for quantification. The regression analysis of response area and injected amount within the above range showed a linear relationship with a coefficient of determination (R^2) ranging from 0.9989 to 0.9995. The purity of each compound in extracts was verified on the basis of the spectroscopic properties of each peak using isoabsorbance plot or three-dimensional graphic and peak purity analyses provided with the ChemStation software. Peak purity analysis allows the spectrum of the identified compounds to be identified and confirmed and to determine whether interference occurs. Chemical structures of *all-trans*-lutein, *all-trans*-zeaxanthin, and 13-*cis*-lutein are presented in **Figure 1**.

Confirmation of the identity of carotenoids was carried out by LC-MS (Thermo Finnigan, San Jose, CA) equipped with a SpectraSystem UV6000-LP ultraviolet detector scanning from 190 to 800 nm and an LCQ Deca ion trap mass spectrometer operated in the atmospheric pressure chemical ionization positive (APCI +ve) mode. The LC-MS separation conditions were as outlined by Young et al. (*17*). **Figure 2** shows typical mass spectra of luetin and zeaxanthin. Although lutein and zeaxanthin have similar molecular weights and structures (**Figure 1**), they exhibited different mass spectra (**Figure 2**). The predominant ion for lutein was the fragment ion at *m*/*z* 551 compared with the protonated molecular ion at *m*/*z* 569 for zeaxanthin. These features were used to confirm the identities of lutein and zeaxanthin.

Statistical Analysis. Data were subjected to analysis of variance to determine significant differences between products and to regression analysis to measure kinetics of lutein degradation during baking and storage using Minitab software (version 12, Minitab Inc., State College, PA). The data were reported as means ± standard deviation (SD).

RESULTS AND DISCUSSION

Stability of Lutein. Lutein was the main carotenoid found in wheat and accounts for 77−83% of the total carotenoids in relatively high-lutein wheat species such as einkorn, durum, Kamut, and Khorasan (*10*). Einkorn is exceptionally high in lutein in comparison with the other wheat species, and thus in the current study it was used as a base flour to study the stability of lutein in a number of unfortified and fortified bakery products. These products include pan bread, flat bread, cookies, and muffins, which represent a variety of staple foods. In addition,

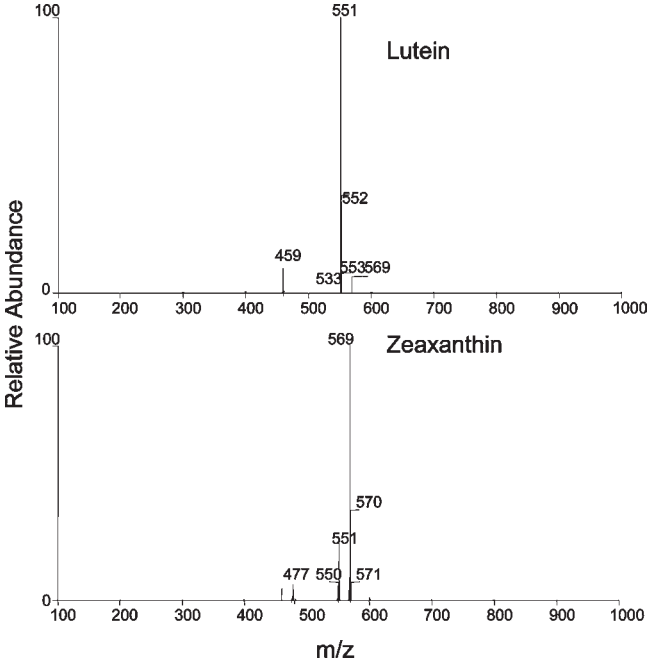

Figure 2. Mass spectra of *all-trans*-lutein and *all-trans*-zeaxanthin.

they are made from different recipes and processing procedures, that is, with or without fermentation, high or no fat, use of baking powder, etc. The stability of lutein in unfortified and fortified einkorn, Khorasan and durum flat breads is presented in **Figure 3**. Baking of flat bread resulted in a significant reduction in *all-trans*-lutein, being about 37−41% for the unfortified breads and 29−33% for the fortified breads. The extent of reduction for natural or added lutein was considerably high and varied slightly among wheat species, einkorn, Khorasan, and durum. The degradation rate of *all-trans*-lutein was concentration dependent. For example, fortified flat bread showed a much greater rate of degradation compared with the unfortified products examined. The degradation of carotenoids is mostly related to their well-known susceptibility

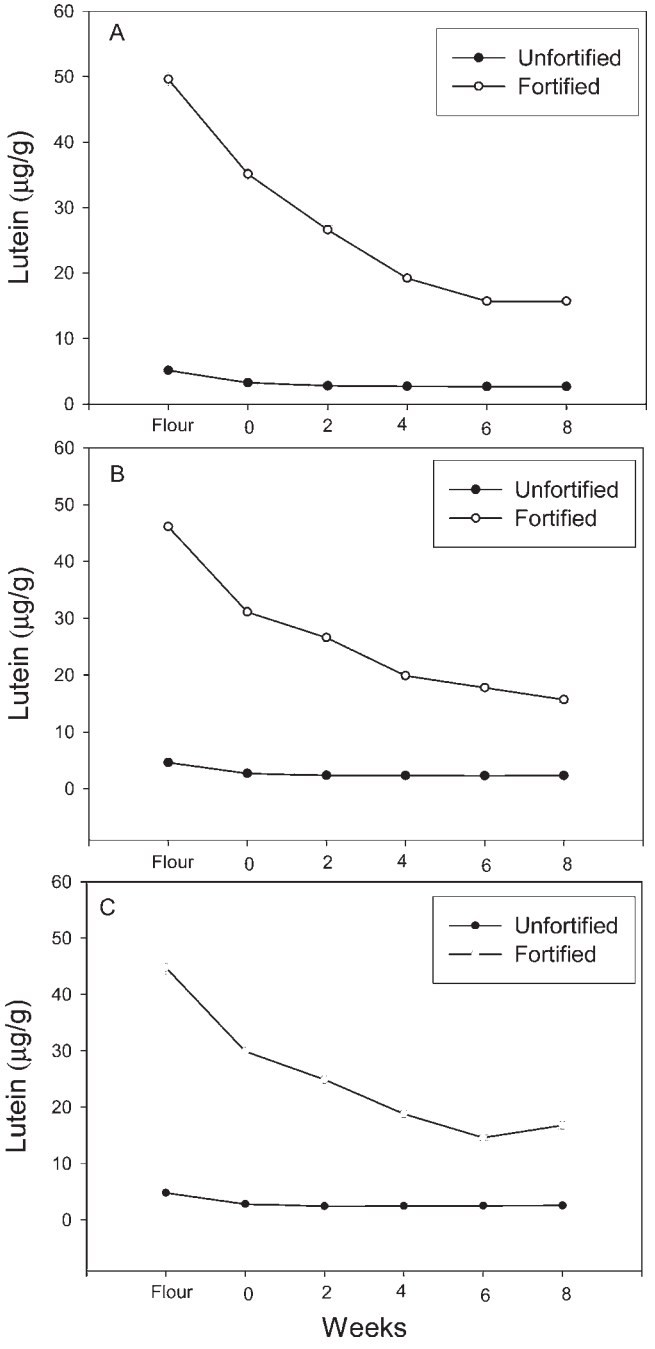

Figure 3. Effects of flat-breadmaking and subsequent storage at ambient temperature on lutein content in unfortified and fortified products: (**A**) einkorn wheat; (**B**) Khorasan wheat; (**C**) durum wheat (standard deviation values presented by error bars).

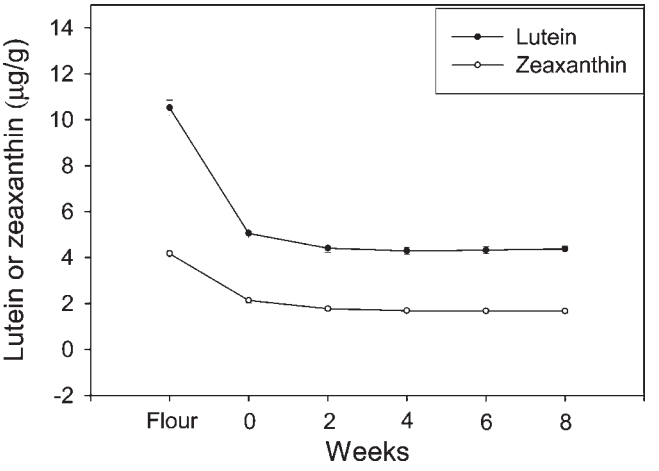

Figure 4. Effects of flat-breadmaking and subsequent storage at ambient temperature on lutein and zeaxanthin content in unfortified flat bread baked from einkorn/corn composite flour (standard deviation values presented by error bars).

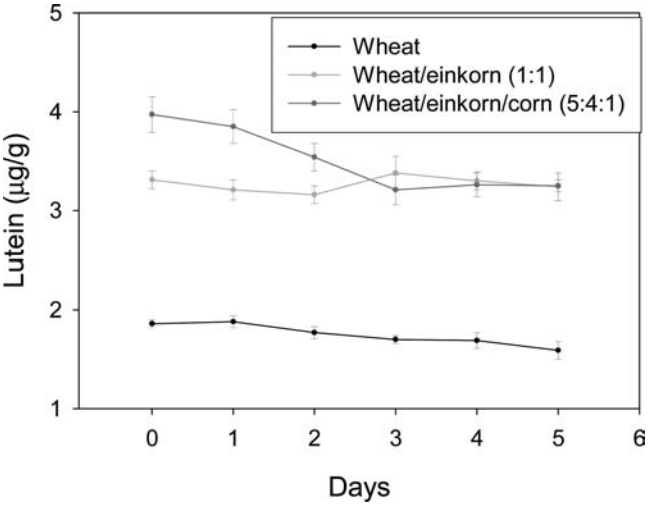

Figure 5. Effects of pan-breadmaking and subsequent storage at ambient temperature on lutein content in unfortified pan bread products (standard deviation values presented by error bars).

to heat (*20*). Storage of flat bread at room temperature for up to 8 weeks had a slight impact on *all-trans*-lutein in the case of unfortified products, whereas the lutein-fortified products showed a linear degradation following first-order kinetics for the fortified flat breads (**Figure 3**). **Figure 4** shows the effects of baking process and subsequent storage on the stability of lutein and zeaxanthin in einkorn/corn flat bread product. Corn was used as high-lutein blending flour due to its high content of lutein and zeaxanthin (*10*, *11*). Because of the high content of lutein in corn flour, flat breads made from einkorn/corn composite flours were evaluated without lutein fortification. Lutein and zeaxanthin are isomers (**Figure 1**); they constitute the pigments found in the yellow spot of the human retina (*5*) and may act similarly in the human body by providing several protective functions. The degradation percentage and rate of lutein on baking were greater than those of zeaxanthin perhaps in this study, but the reason may not be higher concentration of lutein in the composite flour (**Figure 4**). This finding requires further verification using a product containing similar concentrations of both carotenoids. This is the first study to report the stability of lutein in flat bread products, and further research is required to better understand the degradation of lutein and zeaxanthin in these products. Canning of corn in sugar/salt brine solution at 126.7 °C for 12 min did not significantly change the contents of lutein and zeaxanthin in white and golden corn, but α-carotene significantly decreased by about 62% (*21*). However, the study did not measure *cis*-isomers of lutein and zeaxanthin, which were found to increase in canned vegetables (*22*).

Most einkorn cultivars have poor baking quality in pan-breadmaking (*23*), and therefore a blend of bread wheat flour and einkorn flour was used to develop high-lutein pan bread products. The stability of lutein was investigated in two relatively high-lutein pan bread products baked from wheat/einkorn (1:1, w/w) and wheat/einkorn/corn (5:4:1, w/w/w) composite flours and compared with control pan bread made from bread wheat (**Figure 5**).

Corn flour was added to one of the blends to boost carotenoids content, particularly lutein. Lutein content in the two high-lutein pan bread products dropped slightly (**Figure 5**) compared with flat breads (**Figure 4**). The small reduction in lutein in pan bread could possibly be because of the lower concentration of lutein in the baking formulas to which no lutein was added. They were also made from different recipes and baking processes. Hidalgo et al. (*24*) showed carotenoid losses of 21 and 47% for bread crumb and crust, respectively. Bread leavening had almost negligible effects on carotenoids losses, whereas baking resulted in a marked decrease in carotenoids. In pasta, the longer kneading step had significant effects on carotenoid losses, whereas the drying step did not induce significant changes (*24*). Lipoxygenase was found to play a considerable role in the stability of lutein/carotenoids during dough-making, for which a positive correlation was found between carotenoid losses and lipoxygenase activity (*25*). The degradation rate of lutein loss in pan bread was much higher in the high-lutein pan bread compared with the control bread. This indicates lutein degradation kinetics is concentration dependent. Storage of pan bread at room temperature for up to 5 days resulted in an additional decrease in lutein to some extent depending on the base composite flour. Pan bread made from wheat einkorn/corn blend had a slightly higher degradation rate as compared to wheat/einkorn/corn blend. Storage of einkorn flour and bread at various temperatures (−20, 5, 20, 30, and 38 °C) for up to 239 days had major effects on carotenoid degradation, and it was influenced by temperature and time following first-order kinetics (*26*).

Several studies have shown that einkorn flour is well suited for soft wheat applications such as cookies and pastries rich in carotenoids (*9*, *23*, *27*). In the current study, einkorn alone or in a blend with corn flour either unfortified or fortified with lutein was processed into cookies to study the stability of lutein and zeaxanthin. The stability of lutein and zeaxanthin in cookies made from einkorn, einkorn/corn blend (1:1, w/w), and control pastry flour is presented in **Figure 6**. A marked decline occurred in lutein for the fortified einkorn and control cookies, whereas a moderate drop was observed for the unfortified einkorn cookies (**Figure 6A**). The percent of lutein reduction, however, was consistent at 62, 65, and 63% for unfortified einkorn, fortified einkorn, and fortified control cookie, respectively. The degradation rate is dependent on the concentration of lutein as well as baking recipe. The high decline in lutein in cookies compared with bread could be due to the high fat content in the baking recipe that may make lutein and other carotenoids more soluble and exposable to oxidation and isomerization. Cookies made from einkorn and corn composite flours, and fortified with lutein, also exhibited a sharp decline in lutein during baking process, whereas the corresponding unfortified ones had lutein reduction at a lower rate (**Figure 6B**). Zeaxanthin level also reduced on baking, but at much lower rate compared with lutein, perhaps due to its lower concentration in the baking formula. Water biscuit made without adding fat and nonfat dry milk to avoid interferences with the lipophilic oxidation mechanism had lower carotenoid degradation at 31% (*24*). Storage of cookies for up to 8 weeks at ambient temperature produced almost no effect on lutein or zeaxanthin (**Figure 6**). It was noticeable that effects of baking and storage on zeaxanthin degradation losses in the unfortified or fortified cookies were fairly similar. This could indicate that added lutein has no interaction with zeaxanthin during processing and storage (**Figure 6B**).

Muffins were made from a blend of einkorn and corn flours without or with lutein fortification to investigate the effects of baking and storage on lutein in these products. Again, lutein-fortified muffins showed a noticeable decrease in lutein similar to fortified cookies (**Figure 7**). The muffin recipe also contains a high

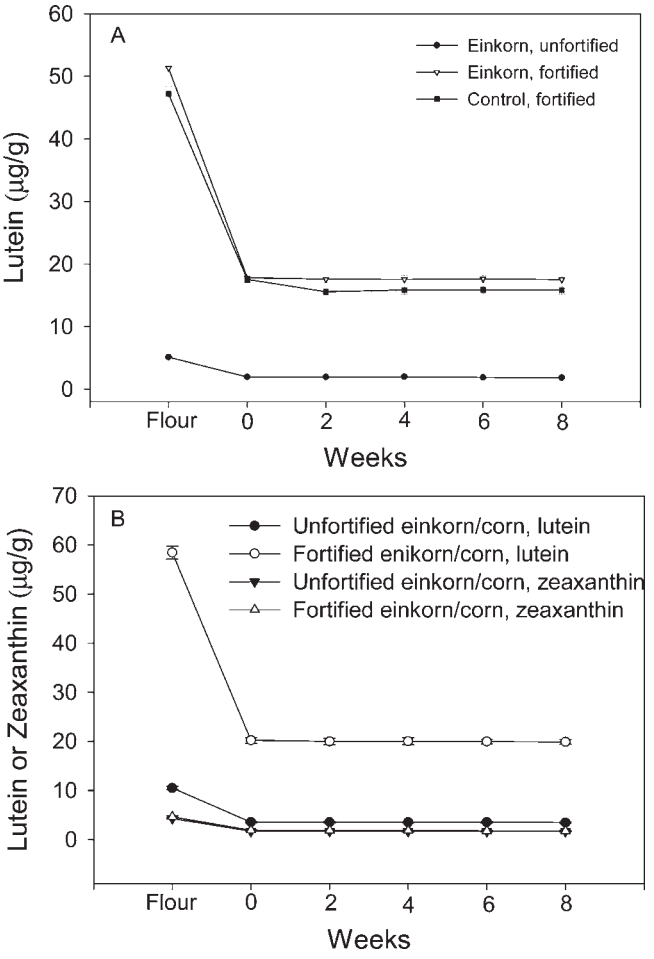

Figure 6. Effects of baking and subsequent storage at ambient temperature on lutein content in unfortified and fortified cookie products: (**A**) control and einkorn products; (**B**) einkorn/corn blend products (standard deviation values presented by error bars).

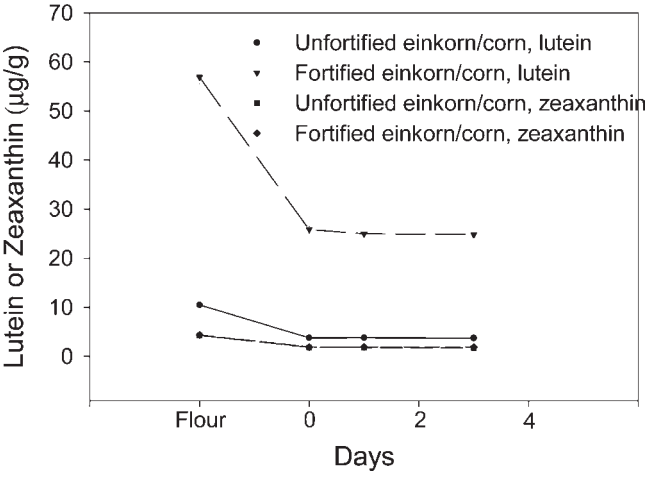

Figure 7. Effects of baking and subsequent storage at ambient temperature on lutein and zeaxanthin content in unfortified and fortified muffin products made from einkorn/corn composite flour (standard deviation values presented by error bars).

percent of fat, which may make lutein more soluble and accessible to processing conditions, causing more degradation by oxidation and isomerization. The reduction percentages for lutein were 64 and 55% in unfortified and fortified muffin, and those for zeaxanthin were 57 and 56%, respectively. This indicates that the

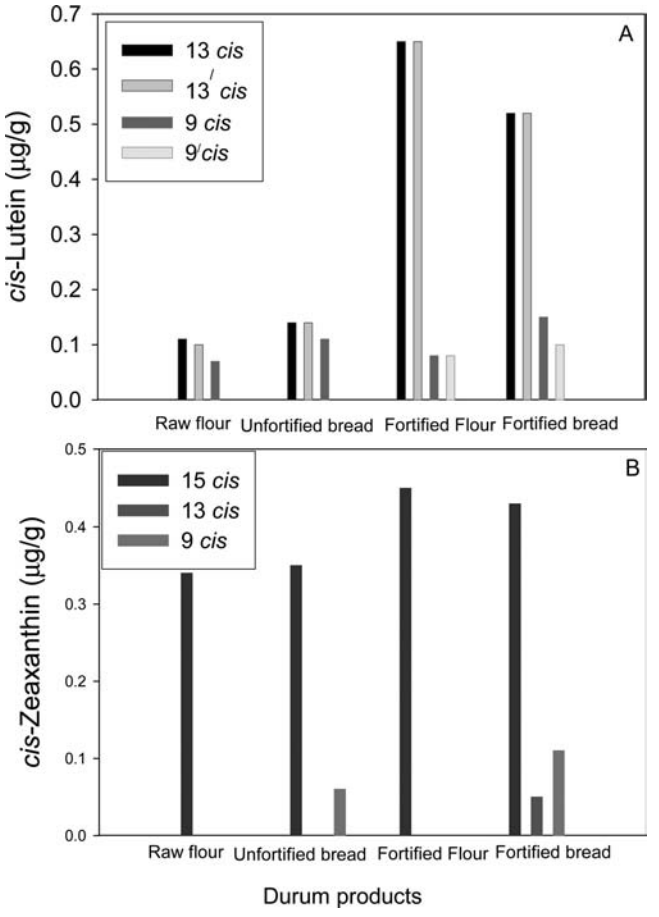

Figure 8. Effects of baking on the formation of *cis*-lutein and *cis*-zeaxanthin isomers in flat bread products: (**A**) lutein; (**B**) zeaxanthin.

extent of reduction or degradation is independent of carotenoid concentration but that the degradation rate is concentration dependent. Storage of muffins for up to 3 days at ambient temperature had no effects on lutein or zeaxanthin content (**Figure 7**). Similar to cookies, zeaxanthin in the unfortified and fortified muffins exhibited identical behavioral effects during baking and storage (**Figure 7**).

Formation of *cis*-Isomers. In wheat and corn flours *all-trans*-lutein is dominant in addition to trace amounts of *cis*-isomers such as 15-*cis*-lutein, 13-*cis*-lutein, 13′-*cis*-lutein, 9-*cis*-lutein, and 9′-*cis*-lutein (*10*, *12*). When lutein-containing products undergo thermal processing or long-term storage (up to a few years), lutein may partially convert into *cis*-isomers. Prolonged storage of wheat flours for up to 5 years resulted in a decrease in carotenid compounds (total, free, mono- and diesterified lutein) at a relative rate similar to the relative rate of oxidation of polyenoic acids in the flours (*28*). *all-trans*- and *cis*-lutein isomers may vary in their bioavailability subject to their solubility in bile acid micelles. For example, *cis*-lycopene is more bioavailable than *trans*-lycopene in vitro and in vivo perhaps because *cis*-isomers are more soluble in bile acid micelles and may be preferentially incorporated into chylomicrometers (*29*). Thus, it is imperative to determine the amount of *cis*-isomers in the end products. The formation of *cis*-lutein and *cis*-zeaxanthin isomers in bread, cookie, and muffin products was assessed during baking and subsequent storage (**Figures 8**−**10**). The formation of 13- and 13′-*cis*-isomers was dominant, and thus their kinetics were investigated in cookies due to their relatively higher content compared with the other products (**Figure 8**). In general 9-, 9′-, 13-, and 13′- *cis*-isomers were detected in the flours and bakery products. **Figure 8** shows

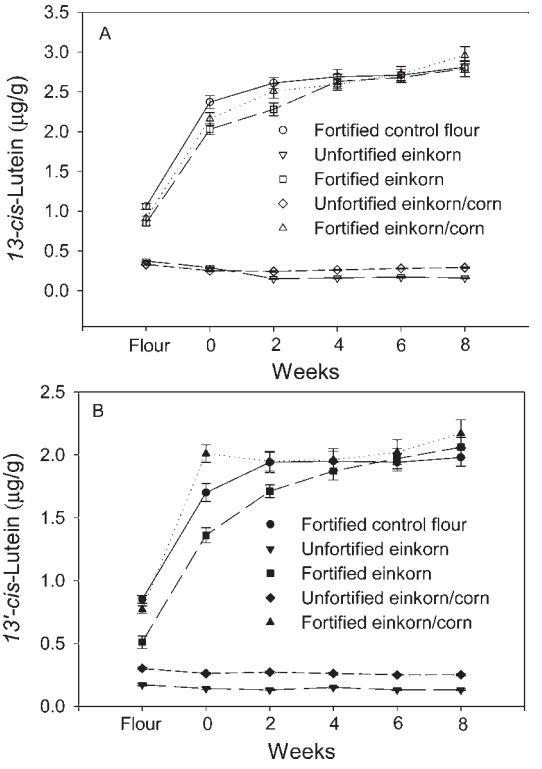

Figure 9. Effects of baking and subsequent storage at ambient temperature on the formation of *cis*-lutein isomers in cookie products: (**A**) 13-*cis*-lutein; (**B**) 13-*cis*-lutein (standard deviation values presented by error bars).

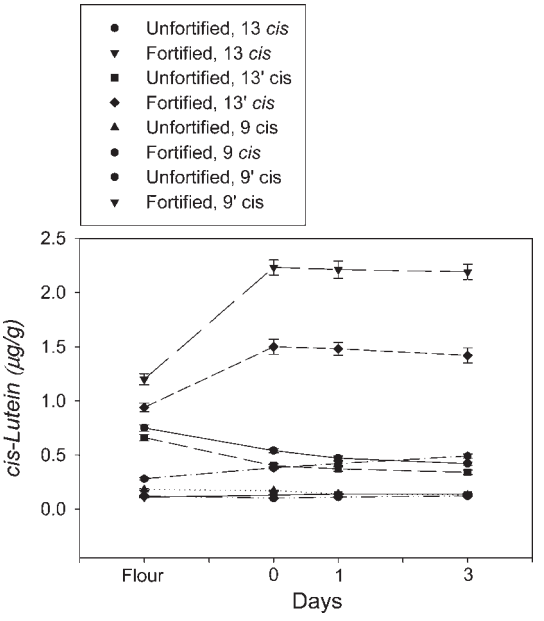

Figure 10. Effects of baking and subsequent storage at ambient temperature on the formation of *cis*-lutein isomers in muffin products (standard deviation values presented by error bars).

the amounts of *cis*-lutein and *cis*-zeaxanthin in durum flat bread. Fortified flours contained higher levels of *cis*-isomers, in particular 13-*cis*-lutein, 13′-*cis*-lutein, and 13-*cis*-zeaxanthin. Small amounts of *cis*-isomers were formed in the unfortified flat bread, whereas *cis*-isomer concentrations in fortified flat breads were lower than in the respective flours. In general, similar concentrations of *cis*-isomers were found in flat bread products.

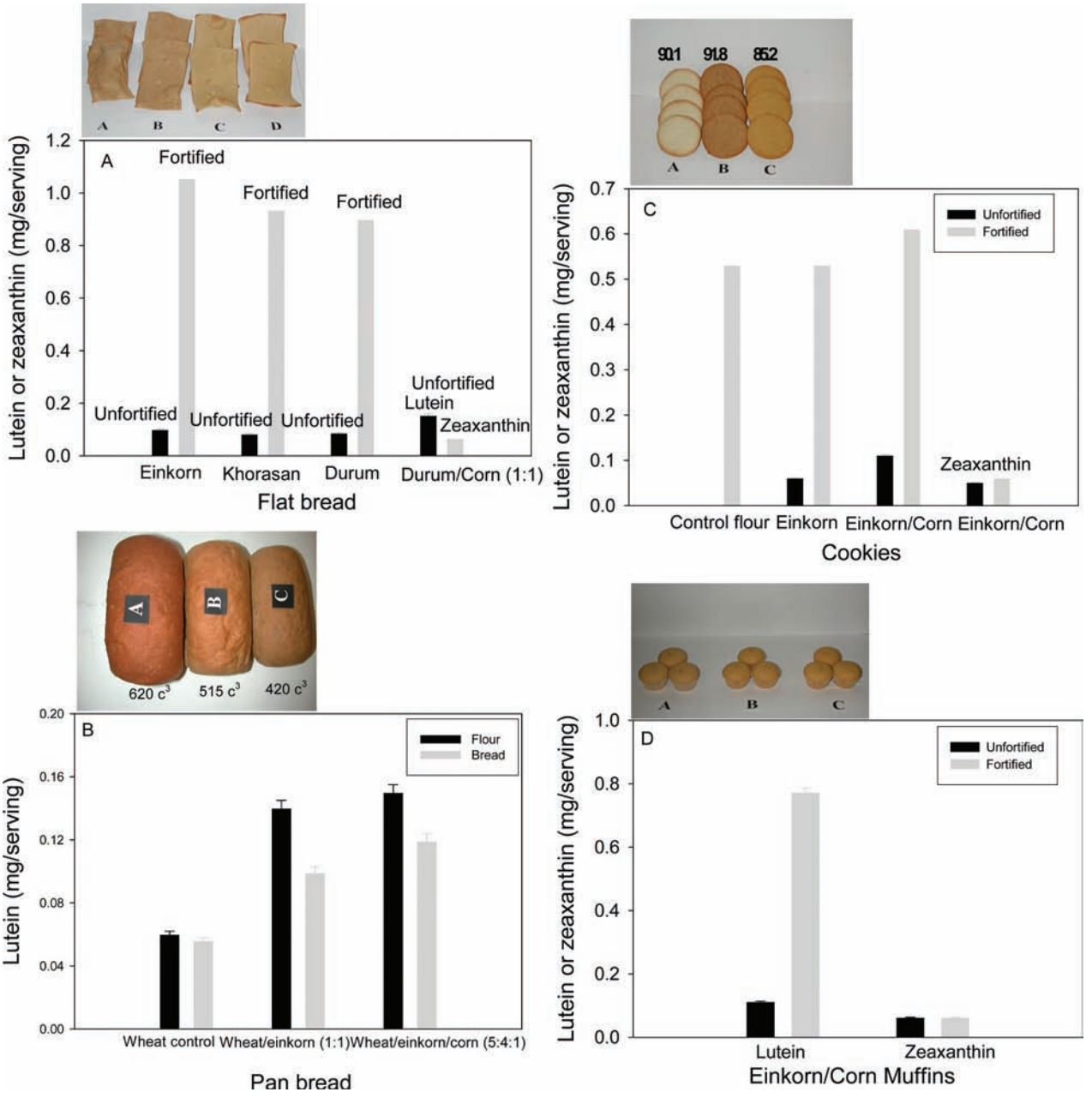

Figure 11. Appearance and lutein content (mg/serving or 30 g) of bakery products: (**A**) flat bread (samples: A, control; B, einkorn; C, Khorasan; D, durum); (**B**) pan bread (samples: A, control; B, control/einkorn; C, control/einkorn/corn); (**C**) cookie (samples: A, control; B, einkorn; C, einkorn/corn); (**D**) muffin (samples: A, unfortified einkorn/corn; B, fortified einkorn/corn; C, fortified einkorn/corn) (standard deviation values presented by error bars).

The formation rates of 13-*cis*-lutein and 13′-*cis*-lutein isomers in cookies made from control flour, einkorn flour, and einkorn/corn composite flour are presented in **Figure 9**, panels **A** and **B**, respectively. On baking fortified cookies, significant amounts (~57%) of *cis*-isomers were formed. However, the unfortified cookies exhibited only slight reduction (~21%) in *cis*-isomer concentrations. The formation of *cis*-isomers in the fortified cookies continued to increase during storage at a roughly linear rate. Storage of unfortified cookies had no effects on *cis*-isomers, with the levels holding virtually steady over 8 weeks.

Muffin products contained several *cis*-lutein isomers ranging from 0.10 to 0.54 μg/g in the unfortified products and from 0.11 to 1.20 μg/g in the fortified products (**Figure 10**). These isomers are 13-*cis*-lutein, 13′-*cis*-lutein, 9-*cis*-lutein, and 9′-*cis*-lutein with 13- and 13′-*cis*-lutein being the dominant ones. As in cookies, fortified muffins had significant amounts of *cis*-isomers formed during baking, in particular 13- and 13′-*cis*-lutein, whereas the concentration of *cis*-isomers in the unfortified cookies dropped slightly during baking. Storage of unfortified and fortified muffins for up to 3 days had no or little effect on the content of *cis*-isomers (**Figure 10**).

Product Quality and Lutein Content. Because the role of lutein in human health has become evident, it is essential to boost the daily intake of lutein, which is low worldwide. For example, the average daily intake of lutein in the United States is about 1.7 mg/day and in Europe is 2.2 mg/day (*16*). These values are below the levels purported to reduce the risk of eye diseases such as cataracts and AMD. The main dietary sources of lutein are dark green vegetables such as spinach and kale or eggs that are consumed in small amounts. Thus, the development of high-lutein staple foods would be of interest to the food industry to enhance lutein intake. Lutein has also been linked with AMD (*1*), and the availability of

Table 1. Average Concentrations (Micrograms per Gram) of Main *all-trans*-Carotenoids in Wholegrain Flours and Lutein-Fortified Wholegrain Flours[a]

	lutein	zeaxanthin	β-cryptoxanthin
cereal flours			
einkorn	5.13 ± 0.21	0.94 ± 0.04	tr
Khorasan	4.65 ± 0.15	0.45 ± 0.02	nd
durum	4.81 ± 0.13	0.47 ± 0.02	nd
wheat	1.11 ± 0.07	tr	nd
corn	22.31 ± 0.77	10.72 ± 0.31	0.95 ± 0.05
fortified cereal flours			
einkorn	49.61 ± 1.51	0.49 ± 0.03	tr
Khorasan	46.14 ± 1.21	0.39 ± 0.03	nd
durum	44.82 ± 1.35	0.42 ± 0.02	nd

[a] nd, not detected; tr, trace amount, <0.05 μg/g.

such high-lutein staple foods would be useful in the management of AMD, in particular for elderly people. Our goal was to develop high-lutein wholegrain foods to enhance the daily intake of lutein as well as to promote the consumption of wholegrain foods. **Figure 11** shows pictures of the high-lutein wholegrain products examined in the current study. It is important to develop a functional food that is palatable and acceptable, so several trials were initially performed to produce quality food prototypes prior to evaluation.

Flat bread is characterized by a rectangular shape and crispy texture with about 3 mm thickness (**Figure 11A**). Overall, the product was acceptable, although it had a bland taste because no flavors were added. Pan bread had loaf volumes ranging from 620 cm^3 for control wheat to 515 cm^3 for wheat/einkorn blend and 420 cm^3 for wheat/einkorn/corn blend (**Figure 11B**). In general, the product was acceptable, but further quality improvements might be required to enhance loaf volume. The einkorn cookie was comparable to the control cookie with regard to spread factor measures, whereas the einkorn/corn cookie had a lower spread factor (width divided by thickness) value of 85.2 compared with 90.1 for control and 91.8 for einkorn cookie (**Figure 11C**). Small-size muffins (each about 30 g) had an appealing appearance and provided a serving portion (**Figure 11D**). Muffin products were acceptable with regard to their appearance, color, taste, and texture. In general, the four bakery products were acceptable and had good appearance. In addition to the quality of the products, the contents of lutein and other carotenoids as well as their bioavailability are important to deliver the expected health benefits of lutein. Other factors such as safety or toxicity of bioactive ingredients need to be considered in the development of functional foods. In the current study we tried to achieve about 1 mg of lutein/serving (30 g) to provide the suggested dose of 5−6 mg per day on the basis of 5−6 servings per day. In addition, if zeaxanthin is taken into consideration as a lutein isomer, the dose would be greater.

Table 1 shows the contents of the main carotenoids, *all-trans*-lutein, *all-trans*-zeaxanthin, and *all-trans*-β-cryptoxanthin, in the wholegrain flours and fortified flours. Fortified wholegrain flours contained approximately 9.3−9.9-fold higher lutein than the unfortified wholegrain flours. **Figure 11** shows the content of lutein as milligrams per serving in the developed products, flat bread (**Figure 11A**), pan bread (**Figure 11B**), cookies (**Figure 11C**), and muffins (**Figure 11D**). Fortified flat bread contained about 0.9−1.1 mg of lutein/serving, whereas the unfortified einkorn had < 0.2 mg/serving. Unfortified einkorn/corn flat breads possessed relatively high levels of lutein per serving but still too low to support the suggested dose (5−6 mg/day). This demonstrates that fortification is necessary to deliver the physiological dose. Other approaches such as developing high-lutein wheat and corn using traditional breeding or more advanced biotechnology could also be employed to increase lutein content in wheat and corn. The unfortified pan bread had relatively small amounts of lutein, about 0.1−0.2 mg/serving. In addition, the lutein einkorn/corn pan bread contained zeaxanthin at about 0.1 mg/serving. Due to the relatively higher loss of lutein in cookies and muffins compared with flat bread, lutein content in fortified cookies was lower than that in flat bread, ranging from 0.5 to 0.6 mg/serving, whereas muffins had a reasonable amount of lutein at 0.8 mg/serving. The unfortified cookies and muffins contained small amounts of lutein, about 0.1 mg/serving. More research is currently underway to determine the bioaccessibility and bioavailability of lutein in vitro in the developed products, bread, cookies, and muffins. Corn-based food products such as bread, porridge, and extruded puff showed various levels of carotenoid bioaccessibility ranging from 48% for porridge to 63−69% for extruded puff and bread (*11*).

In summary, lutein and zeaxanthin are essential dietary components that play significant roles in promoting the health of eyes and skin and in reducing the risk of age-related macular degeneration, cataracts, cancer, and cardiovascular disease. They are sensitive molecules and can undergo oxidation and isomerization when subjected to light, heat, and oxygen. The current study has demonstrated major lutein degradation losses during processing. A fortification approach was used to boost lutein in the end products and to compensate for the losses of lutein on processing and storage. Other approaches may also be required to protect lutein during processing or to develop wheat and corn varieties having higher lutein. Despite the significant losses of lutein during processing, the developed fortified baked products still contain reasonable concentrations (up to 1.0 mg/serving) of lutein and would hold promise for the development of high-lutein functional foods. Further research is underway to evaluate the bioaccessibility and bioavailability of lutein and other important carotenoids in baked products (e.g., breads, cookies, and muffins), that is, how much lutein is transferred from the food matrix into the bile acid micelles and how much lutein is absorbed/passes through intestinal walls. In addition, more research is being carried out to evaluate antioxidant properties of these wholegrain high-lutein food products.

ACKNOWLEDGMENT

We thank Dr. Pierre Hucl from the University of Saskatchewan, Saskatoon, Canada, for providing wheat samples.

LITERATURE CITED

(1) Bone, R. A.; Landrum, J. T.; Mayne, S. T.; Gomez, C. M.; Tibor, S. E.; Twaroska, E. E. Macular pigment in donor eyes with and without AMD: a case-control study. *Invest. Ophthalmol. Vis. Sci.* **2001**, *42*, 235–240.

(2) Olmedilla, B.; Granado, F.; Blanco, I.; Vaquero, M.; Cajigal, C. Lutein in patients with cataracts and age-related macular degeneration: a long-term supplementation study. *J. Sci. Food Agric.* **2001**, *81*, 904–909.

(3) Michaud, D. S.; Feskanich, D.; Rimm, E. B.; Colditz, G. A.; Speizer, F. E.; Willett, W. C.; Giovannucci, E. Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am. J. Clin. Nutr.* **2000**, *72*, 990–997.

(4) Osganian, S. K.; Stampfer, M. J.; Rimm, E.; Spiegelman, D.; Manson, J. E.; Willett, W. C. Dietary carotenoids and risk of coronary disease in women. *Am. J. Clin. Nutr.* **2003**, *77*, 1390–1399.

(5) Krinsky, N. I.; Landrum, J. T.; Bone, R. A. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. *Annu. Rev. Nutr.* **2003**, *23*, 171–201.

(6) Sperling, H. G.; Johnson, C.; Harwerth, R. S. Differential spectral damage to primate cones. *Vis. Res.* **1980**, *20*, 1117–1125.

(7) Olmedilla, B.; Granado, F.; Blanco, I.; Vaquero, M. Lutein, but not α-tocopherol, supplementation improves visual function in patients

with age-related cataracts: a 2-y double-blind placebo-controlled pilot study. *Nutrition* **2003**, *19*, 21–24.
(8) Handelman, G. J. The evolving role of carotenoids in human biochemistry. *Nutrition* **2001**, *17*, 818–822.
(9) Abdel-Aal, E.-S. M.; Young, J. C.; Wood, P. J.; Rabalski, I.; Hucl, P.; Frégeau-Reid, J. Einkorn: a potential candidate for developing high lutein wheat. *Cereal Chem*. **2002**, *79*, 455–457.
(10) Abdel-Aal, E.-S. M.; Young, J. C.; Rabalski, I.; Hucl, P.; Frégeau-Reid, J. Identification and quantification of seed carotenoids in selected wheat species. *J. Agric. Food Chem*. **2007**, *55*, 787–794.
(11) Kean, E. G.; Hamaker, B. R.; Ferruzzi, M. G. Carotenoids bioaccessibility from whole grain and degermed maize meal products. *J. Agric. Food Chem*. **2008**, *56*, 9918–9926.
(12) Hidalgo, A.; Brandolini, A.; Pompei, C.; Piscozzi, R. Carotenoids and tocols of einkorn wheat (*Triticum monococcum* ssp. *monococcum* L.). *J. Cereal Sci*. **2006**, *44*, 182–193.
(13) Leenhardt, F.; Lyan, B.; Rock, E.; Boussard, A.; Potus, J.; Chanliaud, E.; Remesy, C. Genetic variability of carotenoid concentration and lipoxygenase and peroxidase activities among cultivated wheat species and bread wheat varieties. *Eur. J. Agron*. **2006**, *25*, 170–176.
(14) Tsao, R.; Yang, R.; Young, J. C.; Zhu, H.; Manolis, T. Separation of geometric isomers of native lutein diesters in marigold (*Tagetes erecta* L.) by high-performance liquid chromatography−mass spectrometry. *J. Chromatogr., A* **2004**, *1045*, 65–70.
(15) Ma, Q.; Xu, X.; Gao, Y.; Wang, Q.; Zhao, J. Optimization of supercritical carbon dioxide extraction of lutein esters from marigold (*Tagetes erecta* L.) with soybean oil as a co-solvent. *Int. J. Food Sci. Technol*. **2008**, *43*, 1763–1769.
(16) Alves-Rodrigues, A.; Shao, A. The science behind lutein. *Toxicol. Lett*. **2004**, *150*, 57–83.
(17) Young, J. C.; Abdel-Aal, E.-S. M.; Rabalski, I.; Blackwell, B. A. Identification of synthetic regioisomeric lutein esters and their quantification in a commercial lutein supllement. *J. Agric. Food Chem*. **2007**, *55*, 4965–4972.
(18) American Association of Cereal Chemists. *Approved Methods of the AACC*, 10th ed.; St. Paul, MN, 2000; method 14-50.
(19) Abdel-Aal, E.-S. M.; Sosulski, F. W.; Youssef, M. M.; Shehata, A. A. Selected nutritional, physical and sensory characteristics of pan and flat breads prepared from composite flours containing fababean. *Plant Foods Hum. Nutr*. **1993**, *44*, 227–239.
(20) Mercadante, A. Z. Carotenoids in foods: sources and stability during processing and storage. In *Food Colorants Chemical and Functional Properties*; Socaciu, C., Ed.; CRC Press: Boca Raton, FL, 2007; pp 213−240.
(21) Scott, C. E.; Eldridge, A. L. Comparison of carotenoid content in fresh, frozen and canned corn. *J. Food Compos. Anal*. **2005**, *18*, 551–559.
(22) Updike, A. A.; Schwartz, S. J. Thermal processing of vegetables increase cis isomers of lutein and zeaxanthin. *J. Agric. Food Chem*. **2003**, *51*, 6184–6190.
(23) Abdel-Aal, E.-S. M.; Hucl, P.; Sosulski, F. W.; Bhirud, P. R. Kernel, milling and baking properties of spring-type spelt and einkorn wheats. *J. Cereal Sci*. **1997**, *26*, 363–370.
(24) Hidalgo, A.; Brandolini, A.; Pompei, C. Carotenoids evolution during pasta, bread and water biscuit preparation from wheat flours. *Food Chem*. **2010**, *121*, 746–751.
(25) Leenhardt, F.; Lyan, B.; Rock, E.; Boussard, A.; Potus, J.; Chanliaud, E.; Remesy, C. Wheat lipoxygenase activity induces greater loss of carotenoids than vitamin E during breadmaking. *J. Agric. Food Chem*. **2006**, *54*, 1710–1715.
(26) Hidalgo, A.; Brandolini, A. Kinetics of carotenoids degradation during the storage of einkorn (*Triticum monococcum* ssp. *monococcum*). *J. Agric. Food Chem*. **2008**, *56*, 11300–11305.
(27) Borghi, B.; Castagna, R.; Corbellini, M.; Heun, M.; Salamini, F. Breadmaking quality of einkorn wheat (*Titicum monococcum* ssp. *monococcum*). *Cereal Chem*. **1996**, *73*, 208–214.
(28) Farrington, W. H. H.; Warwick, M. J.; Shearer, G. Changes in the carotenoids and sterol fractions during the prolonged storage of wheat flour. *J. Sci. Food Agric*. **1981**, *32*, 948–950.
(29) Boileau, A. C.; Merchen, N. R.; Wasson, K.; Atkinson, C. A.; Erdman, J. W. *cis*-lycopene is more bioavailable than *trans*-lycopene in vitro and in vivo in lymph-cannulated ferrets. *J. Nutr*. **1999**, *129*, 1176–1181.

Received for review June 21, 2010. Revised manuscript received August 9, 2010. Accepted August 11, 2010.